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EXAMINER	
NGUYEN, QUANG	
ART UNIT	PAPER NUMBER
1636	

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13

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/617,116	AGHI ET AL.	
	Examiner	Art Unit	
	Quang Nguyen, Ph.D.	1636	

-- Th MAILING DATE of this communication appears n the cover sheet with the correspondenc address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *20 September 2002* .

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *14-32* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *14-32* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☒ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449) Paper No(s) _____ .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other:

DETAILED ACTION

Applicant's amendment filed on 9/20/02 in Paper No. 12 has been entered.

Claims 1-13 have been cancelled. New claims 14-32 are pending in the present application, and they are examined on the merits herein.

Applicants previously elected prokaryotic vector as a species for a non-viral vector and retrovirus as a species of a viral vector in Paper No. 9.

The text of those sections of Title 35 U.S.C. Code not included in this action can be found in a prior Office Action.

Response to Amendment

The rejection under 35 U.S.C. 112, first paragraph, for lack of Written Description is withdrawn.

The rejection under 35 U.S.C. 103(a) as being unpatentable over Kim et al. as evidenced by Osborne et al. and in view of Garrow et al. and Roy et al is withdrawn in light of Applicants' amendment.

The rejection under 35 U.S.C. 103(a) as being unpatentable over Kim et al. as evidenced by Osborne et al. and in view of Garrow et al., Roy et al. and further in view of Nakanishi is withdrawn in light of Applicants' amendment.

The rejection under 35 U.S.C. 103(a) as being unpatentable over Roy et al. in view of Kim et al., Garrow et al. and Roth et al is withdrawn in light of Applicants' amendment.

Claim Objections

Claim 11 is objected because it contains non-elected embodiments (e.g., an endothelial cell and a macrophage).

Following is a new ground of rejection.

Claim Rejections - 35 USC § 112

Claims 14-32 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for:

A method of enhancing the cytotoxicity sensitivity of neoplastic cells to an antifolate drug, said method comprising: (a) direct innoculation of said neoplastic cells with a vector comprising a DNA sequence encoding folylpolyglutamate synthetase (FPGS) operably linked to a promoter, wherein the FPGS is expressed in the neoplastic cells at a level higher than the endogenous FPGS level of said neoplastic cells; (b) treating the neoplastic cells in step (a) with an antifolate drug that is polyglutamated by said FPGS; whereby the neoplastic cells are killed and wherein said vector is a non-viral vector or a replication defective viral vector;

does not reasonably provide enablement for other embodiments of the claims. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

The factors to be considered in the determination of an enabling disclosure have been summarized as the quantity of experimentation necessary, the amount of direction or guidance presented, the state of the prior art, the relative skill of those in the art, the predictability or unpredictability of the art and the breadth of the claims. *Ex parte Forman*, (230 USPQ 546 (Bd Pat. Appl & Unt, 1986); *In re Wands*, 858 F.2d 731, 8 USPQ 2d 1400 (Fed. Cir. 1988)).

The claims are drawn to a method of enhancing the cytotoxic sensitivity of neoplastic cells, said method comprising: (a) delivering into said neoplastic cells with a vector, said vector comprising a nucleotide molecule encoding FPGS, operably linked to a promoter, wherein said FPGS is expressed in said neoplastic cells at a level higher than the endogenous FPGS level of said neoplastic cells; (b) treating the neoplastic cells of step (a) with an antifolate drug that is polyglutamated by said FDGS; and (c) enhancing the cytotoxic sensitivity of said neoplastic cells to said antifolate; the same method wherein said FPGS is a mammalian FPGS, preferably a human FPGS, or wherein said antifolate drug is methotrexate, edatrexate, aminopterin or a thymidylate synthetase inhibitor or wherein said vector is a viral vector or a non-viral vector, preferably a prokaryotic vector, or wherein said neoplastic cells are breast cancer or colon cancer cells.

The specification teaches by exemplification showing that in comparison with parental 9L rat gliosarcoma cells, 9L/FPGS cells stably transfected with a plasmid vector comprising a human FPGS cDNA are more sensitive to the antifolate drugs such as methotrexate, edatrexate and aminopterin in cell cultures in 4-hour pulses of antifolates. Additionally, the specification teaches

subcutaneous implanted 9L/FPGS tumors in nude mice also respond well to the treatments of methotrexate and edatrexate. Applicants further disclose a bystander killing effect of non-transfected tumor cells was observed in both *in vitro* and *in vivo* resulting from the release of antifolates by transfected tumor cells after the removal of extracellular drugs. The evidence has been noted and considered, however the evidence is not reasonably extrapolated to the instant broadly claimed invention for the following reasons.

When read in light of the specification, the sole purpose for the presently claimed methods is for killing neoplastic cells *in vivo* via the use of folylpolyglutamyl synthetase (FPGS) gene transfer into the neoplastic cells to enhance their cytotoxic sensitivity to an antifolate drug (see page 1, Field of Invention). The nature of the claimed invention falls within the art of *in vivo* gene therapy that remains to be unpredictable for obtaining therapeutic effects at the effective filing date of the present application. Dang et al. (Clin. Cancer Res. 5:471-474, 1999) noted that further advancement in all fields such as gene delivery, gene expression and host immune manipulation is needed to make gene therapy a reality. Dang et al. also pointed out several factors limiting an effective gene therapy, including sub-optimal vectors, the lack of a stable *in vivo* transgene expression, the adverse host immunological responses to the delivered vectors and most importantly an efficient gene delivery to target tissues or cells (last paragraph, col. 2, page 474). The broad claims encompass any route of delivering a vector comprising a nucleotide molecule encoding FPGS into neoplastic cells *in vivo* (including both systemic and local delivery).

However, vector targeting *in vivo* to desired cells or tissues, for this instance neoplastic cells, continues to be unpredictable and inefficient. This is supported by numerous teachings in the art. As examples, Miller & Vile (FASEB 9:190-199, 1995) reviewed the types of vectors available for *in vivo* gene therapy, and concluded that "for the long-term success as well as the widespread applicability of human gene therapy, there will have to be advances ... Targeting strategies outlined in this review, which are currently only at the experimental level, will have to be translated into components of safe and highly efficient delivery systems" (page 198, column 1). Deonarain (Exp. Opin. Ther. Patents 8:53-69, 1998) indicated that one of the main obstacles hampering a successful gene therapy is the "ability to target a gene to a significant population of cells and express it at adequate levels for a long enough period of time." (page 53, first paragraph). Deonarain also reviewed new techniques under experimentation in the art that show promises. One of which is the ligand-targeted receptor-mediated vector approach with a relatively higher level of tissue specificity than viruses can offer. However, this approach to gene therapy is much less efficient than viral gene delivery (column 1, last paragraph, page 65). Verma & Somia (Nature 389:239-242,1997) reviewed various vectors known in the art for use in gene therapy, and the problems that are associated with each. They indicated clearly that resolution to vector targeting *in vivo* had not been achieved in the art (see the entire article). Verma & Somia also discussed the role of the host immune system in inhibiting an efficient targeting of viral vectors to desired cells and tissues (see page 239, and second and third columns of page 242). Verma

& Somia also indicated that appropriate enhancer-promoter sequences can improve expression, but that the "search for such combinations is a case of trial and error for a given cell type." (page 240, sentence bridging columns 2 and 3). The instant specification fails to teach one of skilled in the art how to overcome the unpredictability for *in vivo* vector targeting, such that an efficient transfer and expression of a nucleotide molecule encoding FPGS could be achieved in neoplastic cells *in vivo* through any route of delivery such that upon treatment with an antifolate drug, the drug is activated by the FPGS gene product to effect the killing of said neoplastic cells or enhance the cytotoxic sensitivity of the neoplastic cells. To further support the above cited teachings, more recent reviews on gene directed enzyme/prodrug cancer therapy (Xu et al., Clin. Cancer Res. 7:3314-3324, 2001; Greco et al., J. Cell. Physiol. 187 :22-36, 2001) also note that the lack of an efficient gene delivery to targeted cells still remains a major problem to attain therapeutic efficacy as for all other areas of gene therapy (see the section on GDEPT in Xu et al., and Conclusions in Greco et al.). The exemplification demonstrating the sensitivity of 9L/FPGS rat gliosarcoma cells stably transfected *in vitro* with a plasmid vector comprising a human FPGS cDNA to treatments of antifolate drugs such as methotrexate, edatrexate is not deemed to be sufficient guidance for one skilled in the art for overcoming the unpredictability of *in vivo* vector targeting to attain the contemplated therapeutic effects. As the scope of the instant claims encompasses the use of a non-viral vector including one in the form of a cationic liposome, it is noted that the use of any cationic liposome for systemic or intravenous delivery of a transgene for *in*

vivo applications at the effective filing date of the present application is very limited and unpredictable with regard to obtaining therapeutic effects. Scherman et al. (Current Opinion in Biotechnology 9:480-485, 1998) noted that the use of cationic lipids for *in vivo* gene transfer has remained limited to local administration and that *in vivo* gene delivery by cationic lipids is still found to be an inefficient process as compared to viruses (page 482, col. 1, last paragraph continues to top of col. 2). Romano et al. (Stem Cells 18:19-39, 2000) also noted several obstacles that severely limit the application of cationic liposomes or DNA-protein complexes in therapy, and these include the lack of targeting, low transfection efficiency and the proinflammatory nature of cationic liposomes (Table 1 and page 30, col. 2, middle paragraph). Filion et al. (Int. J. Pharm. 162:159-179, 1998) have even cautioned the use of cationic liposomes for DNA delivery *in vivo* because they elicit a host of adverse effects including a strong anti-inflammatory activity as well as a profound and lethal hypotherma following an oral administration (see page 160, col. 2, last paragraph continues to col. 1 on page 161 and Table 2 on page 168). With respect to other embodiments encompassing the use a DNA-protein complex, a DNA-cationic peptide complex for gene delivery, it is well known in the art that the delivery of a nucleic acid molecule encoding FPGS in such a complex through a systemic delivery would be subjected to degradation by proteolytic enzymes prior to reaching the target cells, in this instance neoplastic cells. Therefore, in light of the totality of the art with respect to *in vivo* vector targeting for achieving the desired therapeutic results, and given the lack of sufficient guidance provided by the present

specification, it would have required undue experimentation for a skilled artisan to make and use the instant broadly claimed invention.

With respect to claims drawn to the elected species of a prokaryotic vector, the claims are not necessarily limited to a live bacterial vector for gene delivery to neoplastic or tumor cells. The claims encompass any prokaryotic vector. The instant specification is not enabled for such a broadly claimed invention because it does not provide any guidance for a skilled artisan on how to make and use any prokaryotic vector to be functional for expressing a transgene in a mammal cell such as a neoplastic cell. For example, how would a prokaryotic vector in the form of a plasmid be functional in a mammalian cell which is contrary to what is known in the art. The present application does not provide any guidance for a skilled artisan on how to make and use such an embodiment. With respect to the scope of the claims encompassing a prokaryotic vector in the form of any live bacterial vector, apart form the cursory mentioning of an attenuated *Salmonella* anticancer vector taught by Pawelek et al. (Cancer Res. 57:4537-4544, 1997), the instant specification fails to provide sufficient guidance for a skilled artisan on how to make and use any prokaryotic cell (both pathogenic and non-pathogenic) as a suitable gene delivery vehicle to neoplastic or tumor cells. There are several issues needed to address, such as whether any prokaryote is capable of invading a neoplastic or tumor cell? Can any prokaryote survive, grow and proliferate in a neoplastic or tumor cell under both anaerobic and aerobic conditions as found within solid tumor? How does any prokaryotic vector specifically target a neoplastic or tumor cell in an efficient

number to mediate the desired results in a subject having a functional immune system, and not in a nude mouse model disclosed by Pawelek et al.? With the lack of sufficient guidance provided by the present application, it would have required undue experimentation for a skilled artisan to make and use the instant broadly claimed invention.

With respect to an embodiment of the claims encompassing the use of replication competent viral vectors and/or pathogenic live prokaryotic vectors for gene delivering into neoplastic cells via a systemic route, neither the instant specification nor the prior art at the effective filing date of the present application teaches the use of replication competent viral vectors such as retrovirus, adenovirus or lentivirus (HIV-1 and HIV-2) or pathogenic live prokaryotic vectors for achieving the contemplated therapeutic results. It is unclear whether the treated individual having neoplastic cells would succumb to the cytotoxic effects of replication competent viral vectors or pathogenic prokaryotes prior to any therapeutic effects contemplated by Applicants could be attained. Pawelek et al. noted that when wild-type *Salmonella* were introduced into melanoma-bearing mice (nude mice), the bacteria found within the tumor at levels exceeding 10^9 per gram, although as pathogens, they caused the death of the mice (see abstract). Hollon, T. (Nature Med. 6 (1): 6, 2000) also reported the first gene therapy death resulting from the utilization of replication-deficient adenovirus in a patient, let alone the use of replication competent HIV or other retroviral viruses. With the lack of guidance provided by the specification regarding to this embodiment of

the claims, it would have required undue experimentation for a skilled artisan to make and use the full scope of the methods as claimed.

With respect to claim specifically encompassing the use of mammalian artificial chromosome as a non-viral gene delivery of FPGS gene for enhancing the cytotoxic sensitivity of neoplastic cells to an antifolate drug, the instant specification fails to provide any specific teachings regarding to the making or using of any mammalian artificial chromosome in a method as claimed. Furthermore, with respect to the issue of mammalian artificial chromosome, Calos (TIG 12:463-467, 1996; PTO-1449, AT2) noted that "a vector of this size is far beyond the size of vectors in current use for gene therapy and poses problems of major dimensions, particularly for the manufacture and delivery of vector DNA. Therefore, while construction of artificial chromosome vectors has not yet been realized, once it is, a series of challenging technical barriers will have to be surmounted before such molecules could reasonably be used as gene therapy vectors" (page 464, col. 2, last paragraph). Therefore, with the lack of guidance provided by the instant specification, particularly in the absence of any *in vivo* example demonstrating an effective use of an artificial chromosome vector comprising a nucleotide sequence encoding FPGS for enhancing the cytotoxic sensitivity to an antifolate drug or killing neoplastic cells *in vivo*, it would have required undue experimentation for a skilled artisan to make and use this particular embodiment of the presently claimed invention.

Accordingly, due to the lack of sufficient guidance provided by the specification regarding to the issues set forth above, the unpredictability of the

gene therapy art, and the breadth of the claims, it would have required undue experimentation for one skilled in the art to make and use the instant broadly claimed invention.

Response to Arguments

Applicants' arguments related to the above rejection in the Amendment filed on 9/20/02 in Paper No. 12 (pages 11-23) have been fully considered. Examiner noted that Applicants presented similar arguments as those in the Amendment filed on 11/29/01 in Paper No. 7 (pages 5-16).

With respect to the issue of the administering route, Applicants mainly argue "the Examiner has not established that the *in vivo* delivery of genetic vectors by methods other than direct injection is of such low efficiency so as to be regarded as impracticable". Applicants further argue that at the filing date of the present application, the scientific literature was replete with examples of successful *in vivo* genetic vector delivery using methods other than direct injection as evidenced by the teachings of Deonarain (page 59, left column), Lan et al/ and Nakanishi. Moreover, Applicants argue that the claims do not require the vector for gene delivery to be delivered into neoplastic cells with any particular minimal level of efficiency, and that successful application of the invention does not require high levels of efficiency of genetic vector delivery. Applicants' arguments are found unpersuasive for the following reasons.

The referred paragraph in the reference of Deonarain merely indicates that liver can take up 85% of the injected DNA molecule via the vascular system.

It is also noted that Deonarain teaches that even in the absence of specific targeting, many molecules can be delivered to the liver since one-fifth of the cardiac output flows through the liver per minute (page 59, bottom of first full paragraph). Deonarain does not teach that the injected DNA molecule could be delivered efficiently to neoplastic cells occurring elsewhere in a patient, for examples in the brain, skin, breast or other organ or tissues to yield the therapeutic effects contemplated by Applicants, particularly the majority of the delivered vector would be sequestered in the liver. It is noted that the instant claims are not drawn to methods of enhancing the cytotoxic sensitivity of neoplastic cells in a liver by intravascular administering a vector comprising a nucleotide molecule encoding FPGS. It should be further noted that the adverse host immune response would further limit the efficiency of neoplastic cells in the liver to be transfected or transformed with the recombinant vector, so that a minimal effective amount of genetically modified neoplastic cells in liver becomes sensitive to an antifole drug and yields a beneficial by-stander effects as contemplated by Applicants. The sentence quoted from the Dang reference "Whereas setbacks in gene therapy were clearly recognized and discussed, there was a unique level of enthusiasm that many of these obstacles could be overcome with meticulously designed basic and clinical studies" does not indicate at all that therapeutic effects could be attained routinely via gene therapy by any routes of delivery, which is contrary to the state of the art exemplified by numerous references cited above. Certainly the instant specification does not provide any guidance, including any relevant in vivo example, to demonstrate

that *in vivo* vector targeting has been overcome to achieve the desired therapeutic effects.

With respect to the cited reference of Lan et al., it is noted that the recombinant adenoviral vector was intraperitoneally injected into nude mice bearing tumor xenografts. The nude mice are known to lack functional immune responses, and therefore they do not represent a typical *in vivo* situation of a patient having neoplastic cells. In a host with a competent immune system, it is well known that the adverse host immune response against recombinant adenoviral vectors would limit an effective amount of transgene to be delivered into desired target cells or tissues to attain contemplated therapeutic outcomes as evidenced by the teachings of Verma et al. discussed above.

With respect to the reference of Nakanishi, the mere statement that "*In vivo* gene transfer is an approach to transfect tissue cells *in situ* by introducing gene transfer vectors through direct injection, through perfusion with catheters, or through an intravenous injection" does not indicate that the unpredictability and inefficient vector targeting *in vivo* to desired cells or tissues known in the art has been overcome, particularly for attaining therapeutic results. Even several years after the publication of the review of Nakanishi, Dang et al. still pointed out the lack of an efficient gene delivery to target tissues or cells still limits the effectiveness of gene therapy (last paragraph, col. 2, page 474). Verma & Somia also indicated clearly that resolution to vector targeting *in vivo* had not been achieved in the art in 1997 (see the entire article), let alone at the time Nakanishi published his review article. Even in an analogous enzyme/prodrug gene therapy

art, recent reviews by Xu et al and Greco et al. also note that the lack of an efficient gene delivery to targeted cells still remains a major problem to attain therapeutic efficacy as for all other areas of gene therapy.

With respect to the cited Roth article, the article clearly states that with respect to clinical applications, the administration of viral vectors to patients is limited to intratumoral delivery since the available vectors have not been approved for systemic administration, and that immune responses against administered vectors limit repetitive administration (page 24, col. 1, last paragraph continues to top of col. 2). Again, indicating that at the effective filing date of the present application, the attainment of therapeutic effects via gene therapy by any route of administration has not been achieved reliably or predictably.

With respect to the adverse host immunological responses to the delivered vectors as a factor limiting an effective gene therapy, Applicants argue that one of the advantages of FPGS gene therapy is that the gene product expressed by the tumor cells is not a foreign enzyme, and therefore it should not elicit an immune response, as might be the case for the expression of a foreign "suicide" gene. Examiner would like to point out that as written the claims encompass encoded FPGS derived from any sources. It has been noted that bacterial FPGS enzymes have limited areas of homology between their sequences and the human FPGS sequence or mammalian FPGS sequence (Garrow et al., Proc. Natl. Acad. Sci. 89:9151-9155, 1992; IDS). As such, such encoded bacterial FPGS would elicit an adverse immune response in a

mammalian host. In additions, the vectors themselves such as adenovirus and any live prokaryotic vector would elicit an adverse host immune response, and thereby limiting the efficiency of the vectors to their intended target cells to yield the desired results.

With respect to Applicants' argument that the claims do not require the vector carrying the FPGS gene be delivered into neoplastic cells with any particular minimum level of efficiency, as long as the cytotoxic sensitivity of the neoplastic cells are enhanced following an antifolate treatment, it should be noted that a minimal threshold level of neoplastic cells must be transfected or transformed with the recombinant vector in order for the genetically modified neoplastic cells become more sensitive to an antifolate drug to yield a beneficial therapeutic effect, such as decreasing a tumor load or growth via the enhanced killing of neoplastic cells directly or indirectly (via an effective by-stander effects).

In light of the state of the gene therapy art at the filing date of the present application, and the absence of sufficient guidance provided by the present application particularly without any *in vivo* example demonstrating that obstacles associated with *in vivo* vector targeting has been overcome, it would have required undue experimentation for a skilled artisan to make and use the methods as claimed.

With respect to the issue of replication competent viral vectors, Applicants argue that "the Examiner has not provided any objective evidence in support of his apparent conclusion that the use of replication competent viral vectors would therefore interfere with the practice of the claimed invention", and that the

presence of inoperative embodiment within the scope of a claim does not render a claim non-enabled. Applicants' arguments are found unpersuasive because Examiner clearly noted that it would have required undue experimentation for a skilled artisan to practice the methods as claimed since neither the instant specification nor the prior art at the effective filing date of the present application teaches a systemic administration of replication competent recombinant viral vectors such as retrovirus, adenovirus or lentivirus (HIV-1 and HIV-2) or any live recombinant prokaryotic cell vector (both pathogenic and non-pathogenic) for achieving any therapeutic results. Applicants have not provided any factual evidence to indicate otherwise. Applicants should be further noted that the scope of the claims must bear a reasonable correlation to scope of enblement provided by the specification as set forth in *In re Fisher*, 166 USPQ 18 (CCPA 1970).

With respect to the issue of mammalian artificial chromosome, Applicants again argue "the Examiner has provided no evidence or arguments to suggest that the reasonable practice of the claimed invention would be substantially impeded due to the presence of mammalian artificial chromosomes within the scope of the claims. Nor has the Examiner set forth any indication that it would require undue experimentation to distinguish the operative from the supposed inoperative embodiments of the claimed invention". Applicants' arguments are found unpersuasive because the instant specification fails to provide any specific teachings regarding to the making or using of any mammalian artificial chromosome for killing a neoplastic cell in a method as claimed, particularly in light of the teachings of Calos (TIG 12:463-467, 1996; PTO-1449, AT2) who

noted that "a vector of this size is far beyond the size of vectors in current use for gene therapy and poses problems of major dimensions, particularly for the manufacture and delivery of vector DNA. Therefore, while construction of artificial chromosome vectors has not yet been realized (**regardless of which methods of construction**), once it is, a series of challenging technical barriers will have to be surmounted before such molecules could reasonably be used as gene therapy vectors" (page 464, col. 2, last paragraph). Even assuming that artificial chromosome vectors can be constructed, there is no factual evidence even in the year 2002 that they can be efficiently transfected or transformed neoplastic cells *in vivo* by any route of delivery to attain the desired therapeutic effects contemplated by Applicants (e.g., killing or enhancing an antifolate drug sensitivity to neoplastic cells). The submitted abstracts of Vos (Curr. Opin. Genet. Devel. 8:351-359, 1998; Cited by Applicants) and Huxley (Gene therapy 1:7-12, 1994; Cited by Applicants) clearly state "Once the size and delivery constraints of human artificial chromosomes (HACs) are circumvented, therapeutic applications will be numerous, particularly for recessive syndromes involving large genes and multigenic diseases" and "Methods which would allow delivery of such large fragments of DNA include liposomes and receptor-mediated uptake, both of which have been shown to work *in vivo*, making such large constructs **potentially applicable** for use in gene therapy", respectively. Clearly, a mammalian artificial chromosome has not been used or applied in a gene therapy for achieving any therapeutic effects at the effective filing date of the present application. In the absence of sufficient guidance provided by the

present application, it would have required undue experimentation for a skilled artisan to make and use the methods as claimed. Applicants are reminded once again that the scope of the claims must bear a reasonable correlation to scope of enblement provided by the specification as set forth in *In re Fisher*, 166 USPQ 18 (CCPA 1970), particularly the claims are drawn to a specific non-enabled embodiment (for this instance the use of a mammalian artificial chromosome in a gene therapy application).

Accordingly, claims 14-32 are rejected under 35 U.S.C. 112, first paragraph, for the reasons set forth above.

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> **The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.**

Claims 14-32 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

In claims 14, 32 and dependent claims of claim 14, the phrase "treating said neoplastic cells" in step b) is unclear. Which neoplastic cells? Neoplastic cells containing a vector comprising a nucleotide molecule encoding FPGS of step a) or non-treated neoplastic cells? Additionally, there is insufficient antecendent basis for the limitation "said neoplastic cell" recited in step c) of the claim. Neoplastic cells are previously recited in the claim, not neoplastic cell. The metes and bounds of the claims are not clearly determined.

Claim 28 recites the limitation "said vector for gene delivery" in line 1 of the claim. There is insufficient antecedent basis for this limitation in the claim. There is no recitation for a vector for gene delivery in claim 14 from which claim 28 is dependent upon. Furthermore, it is unclear how a vector comprising a nucleotide molecule encoding FPGS as recited in claim 14, and at the same time the vector is a cationic peptide (made up of amino acid residues), a starburst polyamidoamine dendrimer, a cationic liposome or a fusogenic liposome as recited in claim 28. Clarification is requested.

Claims 20, 21 and their dependent claims recite the limitation "said chemotherapeutic agent" in lines 1-2 of the claims. There is insufficient antecedent basis for this limitation in the claim. There is no recitation of chemotherapeutic agent in claim 16 from which these claims are dependent upon. The metes and bounds of these claims are not clearly determined.

Claim Rejections - 35 USC § 103

Claims 14-19, 22, 25-28, 31 and 32 are rejected under 35 U.S.C. 103(a) as being unpatentable over Moscow et al. (U.S. Patent 5,763,216) in view of Roy et al. (J. Biol. Chem. 272:6903-6908, 1997; IDS); Kim et al. (J. Biol. Chem. 268:21680-21685, 1993; PTO-1449, AS) and Garrow et al. (Proc. Natl. Acad. Sci. 89:9151-9155, 1992; PTO-1449, AR).

With respect to the enabled scope of the presently claimed invention and the elected species, Moscow et al teach a method of inhibiting the growth of a tumor in a mammal comprising the steps of administering directly into

methotrexate-resistant, transport-deficient cancer cells a vector containing the gene encoding a human reduced folate carrier (RFC), and administering methotrexate to the mammal to enhance the efficacy of traditional methotrexate chemotherapy (see Summary of the Invention, particularly section 3 in cols. 6-9). Moscow et al. teach that appropriate viral vectors such as retrovirus vectors, adenovirus vectors and adeno-associated virus vectors can be used to deliver the gene encoding RFC into a MTX-resistant, transport-deficient cancer cell (col. 7, lines 18-24). Additionally, other recombinant vectors containing the gene encoding RFC can be achieved by any of the methods well-known in the art (col. 6, lines 45-54). Moscow et al. further teach that a short-coming of MTX (a folate antagonist) drug therapy is that previously responsive tumors (e.g. non-Hodgkin's lymphoma, child-hood acute lymphoblasti leukemia, osteosarcoma and breast cancer) can become refractory to MTX after continued exposure. Resistance to MTX in *in vitro* models can result from over-expression of the target enzyme dihydrofolate reductase, alteration of dihydrofolate reductase affinity for MTX, decreased folylpolyglutamate synthase (FPGS), and decreased thymidylate synthase levels, as well as decreased MTX uptake in MTX-resistant cell lines such as murine L1210 leukemia cell lines, human leukemia cell lines, CHO cells and human ZR-75-1 breast cancer cells (col. 1, lines 10-23; lines 47-56).

Moscow et al. do not specifically teach the use of a vector comprising a nucleotide molecule encoding folypolyglutamyl synthase (FPGS) into neoplastic cells to enhance the cytotoxic sensitivity of the neoplastic cells to an antifolate drug such as methotrexate, edatraxate or others.

However, at the effective filing date of the present application, Roy et al. also teach that a major limitation for cancer therapy with classical folate analogues is the acquired resistance of tumor cells to methotrexate and folate analogues (page 6903, col. 2, first full paragraph). Specifically, Roy et al. teach that *in vitro* L1210 tumor cells resistant to methotrexate have a decrease in the rate of FPGS mRNA transcript formation, resulting in lower FPGS activity (page 6907, col. 2, first full paragraph), and that L1210 tumor cells resistant to edatrexate have constitutively down-regulated steady state levels of FPGS or FPGS activity compared with parental L1210 tumor cells and that FPGS mRNA from the variant cells was significantly less effective in mediating formation of the FPGS peptide product in a manner correlating with the FPGS activity or protein (see abstract). Roy et al. further teach that resistance to classical folate analogues in the murine tumor resulting in lower FPGS activity can occur from both transcriptional and post-transcriptional alterations of FPGS gene expression (page 6907, col. 2, first full paragraph). Additionally, at the effective filing date of the present invention, Kim et al. also teach that FPGS-deficient mutant Chinese hamster ovary (CHO AUXB1) cells expressing human folylpolyglutamate synthetase (FPGS) metabolize methotrexate (MTX) to polyglutamates characteristics of human cells (see Table I, page 21681), and that upon a short term exposure to MTX (4 h or 72 h), cells expressing higher levels of human FPGS are more sensitive to the cytotoxicity of MTX compared to cells expressing lower levels of human FPGS (see Table III, page 21682). Kim et al. note that the ability of cells to metabolize MT to longer chain length derivatives enhances

cytotoxicity when MTX is infused for a limited period and then removed, which mimics clinical usage, and that larger effects of FPGS activity levels on the cytotoxicity of antifolates that require polyglutamylation for effective inhibition of target enzymes were also observed (page 21683, col. 2, last paragraph). Kim et al. further teach that lowered FPGS activity may be a general mechanism by which human leukemia cells can become resistant to a wide range of antifolates (page 21684, col. 1, top 5 lines) and that decreased polyglutamylation as a mechanism for inherent MTX resistance for a number of sarcoma and squamous carcinoma cell lines even though FPGS levels appear normal (page 21684, col. 1, bottom of the second paragraph). Garrow et al. already teach the cloning of a human cDNA sequence encoding for FPGS, as well as the expression of human FPGS into the same CHO AUXB1 cells using a plasmid vector pSVK-hFPGS (see Fig. 1 and page 9152, col. 2, first full paragraph).

Accordingly, at the time of the instant invention it would have been obvious and within the scope of skills for an ordinary skilled artisan to modify the method of Moscow et al. by direct delivery of a non-viral (plasmid) or viral vector comprising a DNA sequence encoding human FPGS into neoplastic cells *in vivo* that have acquired resistance to methotrexate and other classical folate analogues in order to reverse the resistance of MTX or other antifolate drugs in these neoplastic cells, so that to enhance the efficacy of conventional anti-folate drug therapy in light of the teachings of Roy et al., Kim et al. and Garrow et al. It is noted that as defined by the present application, a neoplastic cell is a cell whose normal growth control mechanism is disrupted thereby providing the

potential for uncontrolled proliferation (see specification, page 13, lines 4-6). As such, tumor cells resistant to MTX or other antifolate drugs would be encompassed within the scope of neoplastic cells of the instant invention. Furthermore, by reversing the resistance to MTX and other antifolate drugs in the tumor cells, the cytotoxic sensitivity of the tumor cells to an antifolate drug is in effects enhanced.

One of ordinary skilled in the art would have been motivated to carry out the above modification because Moscow et al., Roy et al. and Kim et al. recognize that decreased folylpolyglutamate synthetase is a factor contributing to the resistance of tumor cells to methotrexate or other antifolate drug treatment, and by increasing the exogenous expression of FPGS in MTX or other antifolate resistant tumor cells, the sensitivity to antifolate drugs of the treated tumor cells would be enhanced and thereby enhancing the efficacy of traditional antifolate chemotherapy. One of an ordinary skilled artisan would have a reasonable expectation of success because Kim et al. clearly teach that lowered FPGS activity and decreased polyglutamylation of antifolates are thought to be general mechanisms by which cancer cells become resistant to a wide range of antifolates, and that FPGS-deficient mutant Chinese hamster ovary (CHO AUXB1) cells expressing high levels of human folylpolyglutamate synthetase (FPGS) are more sensitive to the cytotoxicity of MTX compared to cells expressing lower levels of human FPGS (see Table III, page 21682). Furthermore, Roy et al. clearly show that L1210 tumor cells resistant to methotrexate or edatrexate have lowered FPGS activity.

Therefore, the claimed invention as a whole was *prima facie* obvious in the absence of evidence to the contrary.

Claims 14 and 28-29 are rejected under 35 U.S.C. 103(a) as being unpatentable over Moscow et al. (U.S. Patent 5,763,216) in view of Roy et al. (J. Biol. Chem. 272:6903-6908, 1997; IDS); Kim et al. (J. Biol. Chem. 268:21680-21685, 1993; PTO-1449, AS) and Garrow et al. (Proc. Natl. Acad. Sci. 89:9151-9155, 1992; PTO-1449, AR) as applied to claims 14-19, 22, 25-28, 31 and 32 above, and further in view of Pawelek et al. (Cancer Res. 57:4537-4544, 1997).

The combined teachings of Moscow et al., Roy et al., Kim et al., and Garrow et al. have been discussed above. However, none of the references specifically teaches the use of an attenuated live bacterial vector containing a nucleotide molecule encoding FPGS for enhancing the cytotoxic sensitivity of neoplatic cells to an antifolate drug.

However, at the effective filing date of the present application Pawelek et al. teach the use of an attenuated Salmonella as an anticancer vector for gene delivering into tumor cells (see abstract).

Accordingly, it would have been obvious and within the scope of skill for an ordinary skilled artisan to modify the combined teachings of Moscow et al., Roy et al., Kim et al., and Garrow et al. for delivering a DNA sequence encoding human FPGS into neoplastic or tumor cells resistant to methotrexate and other folate analogues by using an attenuated Salmonella as an anticancer gene delivery vehicle or vector as taught by Pawelek.

One of ordinary skilled artisan would have been motivated to carry out this modification because an attenuated Salmonella has been demonstrated to be an effective anticancer gene delivery vector by Pawelek et al., and that the attenuated Salmonella can proliferate and invade mammalian cells under both aerobic and anaerobic conditions such as found in solid tumors (page 4543, col. 2 bottom of the second paragraph).

Therefore, the claimed invention as a whole was *prima facie* obvious in the absence of evidence to the contrary.

Conclusions

No claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Quang Nguyen, Ph.D., whose telephone number is (703) 308-8339.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's mentor, Dave Nguyen, may be reached at (703) 305-2024, or SPE, Irem Yucel, at (703) 305-1998.

Any inquiry of a general nature or relating to the status of this application should be directed to Patent Analyst, Tracey Johnson, whose telephone number is (703) 305-2982.

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